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(71) Applicant: WASHINGTON UNIVERSITY [US/US]; One Brookings Drive, St. Louis, MO 63130 (US).

(72) Inventor: KRAUSE, James, E. ; 7146 Waterman Avenue, University City, MO 63130 (US).

(74) Agent: McBRIDE, Thomas, P.; Rogers, Howell & Haferkamp, 7777 Bonhomme, Suite 1700, St. Louis, MO 63105 (US).

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(54) Title: HUMAN SUBSTANCE P RECEPTOR

(57) Abstract

The disclosure describes the isolation, characterization and cDNA coding the human substance P receptor, the primary structure of the receptor protein, and a CHO cell line transformed with a DNA expression vector containing a cDNA encoding the human substance P receptor.

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HUMAN SUBSTANCE P RECEPTOR

Background of the Invention

This invention relates to the human substance P receptor. More particularly, the invention concerns the molecular cloning and functional expression of the human substance P receptor and a novel stable cell line that expresses large numbers of the recombinant substance P receptor.

Substance P (SP) is a peptide neurotransmitter and neuromodulator originally detected in 1931 based on its smooth muscle contractile activity (1). In 1971 it was isolated based on its sialagogic activity, and its primary structure was established as Arg-Pro-Lys-Pro-Glu-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (2) [SEQ ID NO:1]. SP has since been shown to participate in the regulation of diverse biological activities (3,4,5), and it is an excitatory agent released from central, peripheral and gastrointestinal neurons. In addition, SP regulates certain endocrine and exocrine gland secretions, it aids in the regulation of blood pressure by acting at both central and peripheral sites, and it has been suggested to be involved in the regulation of some immunological disorders and certain inflammatory states. It is now well established that the biological actions of SP are mediated largely via a receptor that interacts specifically with the conserved tachykinin carboxyl terminal domain. The specific amino terminal sequences of the mammalian tachykinin peptides dictate receptor affinity and selectivity. Ligand interaction with the SPR activates guanyl nucleotide binding protein dependent second messenger systems that mediate the specific biological response. Recently, Yokota et al. (6) and Hershey and Krause (7) molecularly characterized and functionally expressed the rat SP receptor (SPR),

and established it to be a member of the G-protein coupled receptor superfamily.

References cited in parentheses herein are listed at the end of the specification.

Brief Description of the Invention

In accordance with the present invention a cDNA encoding the novel human substance P receptor has been isolated and characterized, and nucleotide sequence analysis has been used to deduce the primary structure of the receptor protein. The human substance P receptor consists of 407 amino acid residues and is a member of the G-protein coupled receptor superfamily. Comparison of the novel human and the prior art rat substance P receptor amino acid sequences demonstrated that they have a 94.5% identity that is largely evident in transmembrane domains and in intracellular domains.

In accordance with another aspect of the invention, the novel human substance P receptor was transiently expressed from plasmid pM^2hSPR in a COS-7 cell line and showed a Kd for Tyr^{-1}-substance P binding of 0.24 nM. A clonal cell line stably expressing the novel human substance P receptor from plasmid pM^2hSPR was created in a CHO cell background, said cell line being designated herein as CHO-pM^2-hSPR #10. This cell line expresses 500,000 substance P receptors per cell with an affinity of 0.29 nM. A culture of this cell line is on deposit under the Budapest Treaty with the American Type Culture Collection, Rockville, MD, under accession number ATCC CRL 10824.

Although the invention is particularly illustrated by the use of CHO cells (Chinese hamster ovary) and COS-7 cells (monkey kidney, SV40 transformed) which contain the human substance P receptor directed from the expression of the plasmid pM^2hSPR, it will be understood that other conventional cell lines, e.g., murine cells, HeLa cells, canine cells and the like, can

similarly be used as host cells for expression of the substance P receptor.

The pattern of ligand displacement by naturally occurring tachykinin peptide was substance P>>neurokinin A>neurokinin B. Ligand stimulation of transfected cells results in a rapid and transient inositol 1,4,5-triphosphate response. RNA blot hybridization and solution hybridization demonstrated that the naturally expressed human substance P receptor mRNA was about 4.5 Kb in size, and was expressed in IM-9 lymphoblast and U373-MG astrocytoma cells, as well as in spinal cord and lung but not in liver. These results demonstrate that the human substance P receptor is expressed in many places and it mediates the many diverse functions of human substance P.

Cell lines containing the human substance P receptor cDNA are useful for examining cellular mechanisms regulating human substance P receptor mRNA expression and for screening for antagonists of human substance P such as may be useful for central, peripheral and gastrointestinal system disorders, inflammation and immune disorders. Since tissues in the human body that express the substance P receptor express only about 5-10,000 receptors per cell, the substantially and significantly higher expression system of about 500,000 receptors per cell in accordance with the present invention permits rapid and faster screening of candidate compounds acting at the substance P receptor. The human substance P receptor also is useful as a diagnostic approach for identifying abberrant receptor sequences in human disease states.

Detailed Description of the Invention

While the specification concludes with claims particularly pointing out and distinctly claiming the subject matter regarded as forming the present invention, it is believed that the invention will be

better understood from the following detailed description of preferred embodiments of the invention in conjunction with the appended drawings, in which briefly:

FIG. 1 shows the nucleotide sequence and deduced amino acid sequence [SEQ ID NO:3] of the human substance P receptor. Nucleotide numbering shown on the right side starts with +1 beginning with A of the initiator methionine codon. Amino acid sequence is numbered below the displayed sequence. The putative α-helical transmembrane domains labeled MI-MVII are underlined.

FIG. 2 shows the expression of the human substance P receptor in COS-7 cells in two bar graph panels. A. Comparison of ^{125}I-Tyr^{-1}-SP binding to nontransfected cells and to cells transfected with a plasmid encoding either the human SPR, the human SPR in the antisense orientation or the rat SPR. B. Competition of ^{125}I-Tyr^{-1}-SP binding by naturally occurring and synthetic tachykinin peptides. Transfection conditions and ligand binding were performed with 0.1 nM ^{125}I-Tyr^{-1}-SP as described in Methods hereinbelow. Each datum represents the X ± SEM of four duplicate determinations performed with different preparations of transfected cells.

FIG. 3 is a graphical representation which shows the displacement of ^{125}I-Tyr^{-1}-SP binding to transfected COS-7 cells by the naturally occurring tachykinins: substance P, neurokinin A and neurokinin B. Transfection conditions and ligand binding were performed with 0.1 nM ^{125}I-Tyr^{-1}-SP as described in Methods hereinbelow. Each datum point represents the X± of four determinations performed in duplicate. The SEM was less than 5% for all data presented.

FIG. 4 is a graphical representation which shows the saturation analysis of ^{125}I-Tyr^{-1}-SP binding to transfected COS-7 cells. Cells were transfected and ligand binding was performed as described in Methods hereinbelow. The data shown are from four determinations performed in duplicate on separate transfected cell preparations. The variation in ligand concentration for all data points was less than 3% of the mean concentration shown. The calculated Kd and β_{max} values were 0.24± and 151,000± per cell, respectively.

FIG. 5 is a graphical representation which shows a human substance P stimulated inositol trisphosphate response as a function of time after stimulation of transfected COS-7 cells. Cells were transfected, harvested and stimulated with 1 μM human substance P, and inositol trisphosphate levels were determined as described in Methods hereinbelow. The data shown represents the results from a single transfection and stimulation test. Similar results were obtained in a repeat of the test.

FIG. 6 shows the analysis of human substance P receptor mRNA expression patterns by RNA blot and solution hybridization methods. The upper left shows the RNA blot results, the upper right shows the solution hybridization-nuclease protection results, and the lower portion illustrates the probes used. For RNA blots, 2 μg poly(A)$^+$ RNA was denatured, electrophoresed on 1% gels and transferred to nitrocellulose as described in Methods hereinbelow. For solution hybridization, 25 μg total RNA was annealed with the coding region probe, and non-hybridized probe was digested with RNases A and T, as described in Methods hereinbelow. An autoradiogram of protected species after electrophoresis on a denaturing 6% polyacrylamide gel is shown. Standards for the RNA blot were 0.24 to 9.5 Kb RNA ladder (BRL,

Gaithersburg, MD), and standards for the nuclease protection gel were radiolabeled MspI-digested pBR322.

FIG. 7 shows a comparison of the amino acid sequences of human substance P receptor [SEQ ID NO:3] and rat substance P receptor [SEQ ID NO:7]. Identical residues between the two sequences are indicated by the vertical line. Putative membrane spanning domains MI-MVII are overlined. The closed triangles indicate consensus N-linked glycosylation sites, the filled circles indicate potential intracellular serine and threonine phosphorylation sites, and the arrow depicts a potential palmitoylation site.

Standard biochemical nomenclature is used herein in which the nucleotide bases are designated as adenine (A); thymine (T); guanine (G); and cytosine (C). Corresponding nucleotides are, for example, deoxyadenosine-5'-triphosphate (dATP). Amino acids are shown either by conventional three or one letter abbreviations as follows:

Abbreviated Designation		Amino Acid
A	Ala	Alanine
C	Cys	Cysteine
D	Asp	Aspartic acid
E	Glu	Glutamic acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine

In order to illustrate specific preferred embodiments of the invention in further detail, the following exemplary laboratory work was carried out. References to publications cited herein in parenthesis are listed at the end of the specification. Although specific examples are illustrated herein, it will be appreciated that the invention is not limited to these specific examples or the details described therein.

EXAMPLES

Materials and Methods

Materials. Most reagents used herein are conventional and have been described previously (7, 8, 9). The plasmid pM^2 was obtained from Dr. Irving Boime, Washington University School of Medicine (10). Oligonucleotides for sequence analysis were obtained from the Washington University Protein Chemistry Facility. IM-9 immunoblast cells were obtained from Drs. Norman Boyd and Susan Leeman, University of Massachusetts Medical Center, and U373 MG astrocyte cells were obtained from the ATCC (ATCC HTB 17). Tyr^{-1}-Substance P (Tyr-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) [SEQ ID NO:4] was synthesized by the Washington University Protein Chemistry Facility and was purified to homogeneity by HPLC using the general procedures previously described (11). Radioiodination of peptide was performed using the conventional chloramine T oxidative iodination procedure and HPLC purification of the monoiodo form of Tyr^{-1}-SP.

RNA isolation, cDNA and genomic cloning, PCR methods and nucleotide sequence analysis. The methods for RNA isolation, $(poly(A)^+$ RNA selection and cDNA synthesis are conventional and have been described previously (7, 8, 9). PCR was performed using a Perkin Elmer thermal cycler as previously described (7) with IM-9 cDNA as target. Initially a cDNA was generated by PCR using oligonucleotide primers corresponding to G-protein coupled receptor membrane spanning domains II and VII. A 671 bp cDNA was isolated, subcloned into BLUESCRIPT (pBS) and sequenced; it contained an open reading frame with 90.5% identity to the corresponding rat substance P receptor cDNA (6,7) and gene (9) sequence. This plasmid was termed pBS-hSPRII-VII and the inserted cDNA corresponded to nucleotides 237-908 of

that shown in Figure 1. The 5' and 3' extents of the hSPR cDNA coding region as well as nontranslated sequences were determined by isolation and characterization of human SPR genomic exons 1 and 5, respectively, using the pBS-hSPR II-VII cDNA insert and rat genomic exons (9) as probes, and is shown in Figure 1. An amplified human λ Dash II genomic library (Stratagene, La Jolla, CA) was screened and 10 positive phage were isolated and characterized by restriction mapping and hybridization analysis. Hybridizing sequences corresponding to exons 1-5 were identified, and exons 1 and 5 were isolated as 1.2 kb EcoR1 and 1.4 kb EcoR1 fragments, subcloned and sequenced. The predicted coding region of the human SPR was generated by PCR with IM-9 cDNA by using oligonucleotides corresponding to the coding region 5' (5'CCACCATGGATAACGTCCTCCCGGTG 3') [SEQ ID NO:5] and 3'(antisense, 5'CTAGGAGAGCACATTGGAGGAGAA3') [SEQ ID NO:6] ends as primers. The cDNA generated was isolated by agarose gel electrophoresis and was blunt-end ligated into SmaI-digested pBS. Electroporation of bacterial cells with the ligated DNA yielded multiple isolates that were further analyzed by restriction mapping and by nucleotide sequence analysis. One cDNA (corresponding to bases -5 to +1227 of that shown in Fig. 1) was isolated after restriction with HindIII and BamHI (present in the pBS polylinker), and was made blunt-ended with Klenow fragment. The pM^2 was also blunt-ended with Klenow after BamHI digestion. The cDNA was ligated to pM^2, and was used to transform E. coli XL-1 Blue cells by electroporation. Colonies containing inserts were identified and the orientation of inserts was determined by restriction analysis. Two plasmids, called pM^2-hSPR and pM^2-hSPR antisense, were identified. Sequence analysis was performed as described previously (7, 8, 9).

Transfection of COS-7 cells, ligand binding tests and inositol 1, 4, 5 trisphosphate assay. COS-7 cells plated at 50 to 90% confluence were transfected by conventional means as previously described (7). Cells harvested 48-72 hours after transfections were incubated with ^{125}I-Tyr^{-1}-SP (for 2 hours at 4°) and binding was determined by a conventional rapid filtration assay as previously described (11). Typical binding tests were performed with approximately 150,000 transfected cells per assay tube. Competition binding was performed by adding the competitor prior to that of radiolabelled ligand. Ligand binding data was analyzed by the LIGAND program (12). Cellular inositol 1,4,5-trisphosphate level was determined with a radioreceptor assay (13) with rat cerebellar membranes (14) using conventional extraction and assay conditions as previously described (15).

RNA blot and solution hybridizations. These were performed by conventional procedures as described previously (8, 9, 16, 17). A random-primer labeled cDNA was prepared with Klenow fragment of DNA polymerase I for the pBS-hSPRII-VII cDNA insert, and an antisense RNA was prepared by transcription using T_7 RNA polymerase and EcoR1 linearized pBS-hSPRII-VII. RNA gels (1.0%) were blotted onto Nytran membranes, and the protected RNA species from solution hybridization tests were electrophoresed on 6% polyacrylamide gels containing 7M urea. Autoradiography was performed at -70° with an intensifying screen.

RESULTS

A human SPR cDNA fragment corresponding to nucleotides +237 to +908 in Figure 1 was generated by PCR from cDNA prepared from IM-9 lymphoblast cell RNA using conventional procedures previously described (7). The 5' end of the coding region was determined by

isolation and sequence analysis of the human SPR gene exon 1, and the 3' end of the cDNA was determined by isolation and sequence analysis of the human SPR gene exon 5 as described in Methods hereinbefore. These sequences provided the 5' and 3' translated sequences of the human SPR, and a PCR using IM-9 cell cDNA was used to generate a full coding region containing cDNA. This cDNA was subcloned into the pM^2 expression vector in which the cDNA is under the control of the Harvey murine sarcoma virus LTR (10) and was used for functional expression.

COS-7 cells were transfected with pM^2-hSPR, pM^2hSPR antisense and pM^2rSPR, three plasmids that contain the human SPR cDNA, the human SPR cDNA inserted in the antisense orientation and the rat SPR cDNA (7), and 48 to 72 hours later the cells were examined for binding of ^{125}I-Tyr^{-1}-SP using a rapid filtration assay. Figure 2A shows these results in which the human and rat SPR construct transfected cells bind 15,000 to 25,000 cpm ligand that is displaced by 1 μM SP. Nontransfected cells or cells transfected with pM^2hSPR antisense showed no specific binding. Consequently, ligand displacement and saturation analyses were performed with the pM^2hSPR construct to determine whether the binding site corresponded pharmacologically to that of the SPR or so-called NK-1 type tachykinin binding site. Figure 2B shows that at 10 nM SP or physalaemin, specific ^{125}I-Tyr-$^{-1}$-SP binding was reduced by 85 to 95%, whereas 10-fold higher concentrations of tachykinins potent at NK-2 and NK-3 receptors, including neurokinin A, neurokinin B, neuropeptide γ, neuropeptide K, eledoisin and senktide, were much less potent in displacing radiolabelled ligand binding. Also, substance P free acid was much less potent in this regard, thereby demonstrating the importance of the substance P carboxyamide moiety in ligand binding. Additional tests were performed with SP, NKA and NKB at various doses to determine the IC_{50} values for displacing ^{125}I-Tyr^{-1}-SP

binding, and these data are shown in Figure 3. SP was the most potent displacer of ligand binding compared to NKA and NKB, with IC_{50} values of 0.72 ± 0.9 nM, 0.63 ± 0.06 μM, and 1.12 ± 0.21 μM, respectively, each with Hill coefficients of 0.94 - 0.96. Therefore, this cloned human cDNA encodes a sequence in transfected cells that upon ligand binding analysis has the characteristics of a SPR or NK-1 type tachykinin binding site. Saturation analysis of ^{125}I-Tyr^{-1}-SP binding was performed to determine the affinity and relative number of binding sites expressed by pM^2hSPR transfection of COS-7 cells. Figure 4 shows these results; Scatchard analysis of this data by the ligand program (12) provides a 1 site fit with a k_d value of 0.24 ± 0.01 nM, with an average of 151,000 ± 8,000 sites expressed per cell.

Transiently transfected COS-7 cells were stimulated with 1 μM SP to determine cellular inositol-1,4,5 trisphosphate responses. Two tests were performed in which time points after stimulation of 5, 10, 15, 20, 30, 60 and 120 seconds were analyzed. A transient response of 2.5 to 3-fold above resting levels was observed (Figure 5) at 10 to 15 seconds after stimulation with a return to basal level by 20 to 30 seconds.

Some patterns of human SPR RNA expression were also examined using RNA isolated from cell lines or tissues, and these data are shown in Figure 6. By northern blot analysis, a single hybridizing species of approximately 4.5 kb was identified in poly(A)$^+$ RNA isolated from IM-9 cells but not in liver. Similar hybridizing species were observed with both an RNA coding region probe and a DNA probe corresponding to exon I of the SPR gene. The coding region antisense RNA probe was also used in a more sensitive solution hybridization-nuclease protection test in which the probe corresponds to a 712 base sequence which when annealed with hSPR mRNA, will protect a species of 671

bases. Figure 6 shows that this probe will protect a species of 671 bases in IM-9 and U373 cell RNA preparation and also in spinal cord and lung RNA preparations, that correspond to SPR mRNA; these are not observed in HepG2 cell or liver RNA preparations. In IM-9 cell RNA preparations, two additional protected species of approximately 150 bases and 350 bases were noted. These species have not been completely characterized, but they may correspond to exon 1 protected species and exons 1 plus 2 protected species which would be present in partially spliced RNAs. These have been observed in rat tissues (9) and appear to correspond to slowly spliced nuclear SPR RNA precursors.

Figure 7 shows a comparison of primary structures of human and rat SPR protein, as deduced from cDNA cloning and sequence analysis. Twenty-two of the 407 residues are different between the sequences; these differences are distributed throughout and are generally conservative. Both sequences encode receptors with 7 putative α-helical transmembrane domains based on hydrophobicity plotting and by comparisons to other G-protein coupled receptors. The human SPR has 2 N-linked glycosylation sites in the amino terminal domain, and a potential palmitoylation site (cys-323) 15 residues carboxyl terminal to the MVII transmembrane domain. Multiple potential serine and threonine phosphorylation sites exist in the 3rd cytoplasmic and carboxyl terminal domain and many of these are conserved between the two sequences. The carboxyl terminal tail region is separated by an acidic region about half way into the sequence, which separates the two Ser/Thr rich regions.

The foregoing results indicate that the novel human and previously characterized rat (6,7) SPR's show a sequence identity of 94.6% throughout the entire primary structure. Sequences within all 7 putative α-helical transmembrane domains and within many regions of both extracellular and intracellular domains are conserved. These include two consensus N-linked

glycosylation sites in the amino terminal region, a conserved consensus palmitoylation site (Cys-323) carboxyl terminal to transmembrane domain VII, and multiple potential serine and threonine phosphorylation sites on intracellular domains, especially that of the third cytoplasmic domain and in the carboxyl terminal region. Two regions showing divergence are scattered in the amino terminal region and are clustered near the carboxyl terminus within the carboxyl terminal domain. Based upon the high degree of sequence identity it is likely that sequences within the primary structure essential for G-protein coupling, high affinity agonist binding, and desensitization of receptor responses are conserved.

Various other examples will be apparent to the person skilled in the art after reading the present disclosure without departing from the spirit and scope of the invention, and it is intended that all such other examples be included within the scope of the appended claims.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Krause, James E.

(ii) TITLE OF INVENTION: Human Substance P Receptor

(iii) NUMBER OF SEQUENCES: 7

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Scott J. Meyer, Monsanto Co., A3SD
(B) STREET: 800 N. Lindbergh Blvd.
(C) CITY: St. Louis
(D) STATE: Missouri
(E) COUNTRY: U.S.A
(F) ZIP: 63167

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Meyer, Scott J.
(B) REGISTRATION NUMBER: 25,275
(C) REFERENCE/DOCKET NUMBER: 07-24(776)A

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (314)694-3117

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 11
(D) OTHER INFORMATION: /label= amide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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1 5 10

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1766 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 211..1431

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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TAC TCC ATG ACG GCT GTG GCC TTT GAT AGG TAC ATG GCC ATC ATA CAT 618
Tyr Ser Met Thr Ala Val Ala Phe Asp Arg Tyr Met Ala Ile Ile His
                125                 130                 135

CCC CTC CAG CCC CGG CTG TCA GCC ACA GCC ACC AAA GTG GTC ATC TGT 666
Pro Leu Gln Pro Arg Leu Ser Ala Thr Ala Thr Lys Val Val Ile Cys
            140                 145                 150

GTC ATC TGG GTC CTG GCT CTC CTG CTG GCC TTC CCC CAG GGC TAC TAC 714
Val Ile Trp Val Leu Ala Leu Leu Leu Ala Phe Pro Gln Gly Tyr Tyr
        155                 160                 165

TCA ACC ACA GAG ACC ATG CCC AGC AGA GTC GTG TGC ATG ATC GAA TGG 762
Ser Thr Thr Glu Thr Met Pro Ser Arg Val Val Cys Met Ile Glu Trp
    170                 175                 180

CCA GAG CAT CCG AAC AAG ATT TAT GAG AAA GTG TAC CAC ATC TGT GTG 810
Pro Glu His Pro Asn Lys Ile Tyr Glu Lys Val Tyr His Ile Cys Val
185                 190                 195                 200

ACT GTG CTG ATC TAC TTC CTC CCC CTG CTG GTG ATT GGC TAT GCA TAC 858
Thr Val Leu Ile Tyr Phe Leu Pro Leu Leu Val Ile Gly Tyr Ala Tyr
                205                 210                 215

ACC GTA GTG GGA ATC ACA CTA TGG GCC AGT GAG ATC CCC GGG GAC TCC 906
Thr Val Val Gly Ile Thr Leu Trp Ala Ser Glu Ile Pro Gly Asp Ser
            220                 225                 230

TCT GAC CGC TAC CAC GAG CAA GTC TCT GCC AAG CGC AAG GTG GTC AAA 954
Ser Asp Arg Tyr His Glu Gln Val Ser Ala Lys Arg Lys Val Val Lys
        235                 240                 245

ATG ATG ATT GTC GTG GTG TGC ACC TTC GCC ATC TGC TGG CTG CCC TTC 1002
Met Met Ile Val Val Val Cys Thr Phe Ala Ile Cys Trp Leu Pro Phe
    250                 255                 260
```

```
CAC ATC TTC TTC CTC CTG CCC TAC ATC AAC CCA GAT CTC TAC CTG AAG 1050
His Ile Phe Phe Leu Leu Pro Tyr Ile Asn Pro Asp Leu Tyr Leu Lys
265                 270                 275                 280

AAG TTT ATC CAG CAG GTC TAC CTG GCC ATC ATG TGG CTG GCC ATG AGC 1098
Lys Phe Ile Gln Gln Val Tyr Leu Ala Ile Met Trp Leu Ala Met Ser
                285                 290                 295

TCC ACC ATG TAC AAC CCC ATC ATC TAC TGC TGC CTC AAT GAC AGG TTC 1146
Ser Thr Met Tyr Asn Pro Ile Ile Tyr Cys Cys Leu Asn Asp Arg Phe
            300                 305                 310

CGT CTG GGC TTC AAG CAT GCC TTC CGG TGC TGC CCC TTC ATC AGC GCC 1194
Arg Leu Gly Phe Lys His Ala Phe Arg Cys Cys Pro Phe Ile Ser Ala
        315                 320                 325

GGC GAC TAT GAG GGG CTG GAA ATG AAA TCC ACC CGG TAT CTC CAG ACC 1242
Gly Asp Tyr Glu Gly Leu Glu Met Lys Ser Thr Arg Tyr Leu Gln Thr
    330                 335                 340
```

```
CAG GGC AGT GTG TAC AAA GTC AGC CGC CTG GAG ACC ACC ATC TCC ACA 1290
Gln Gly Ser Val Tyr Lys Val Ser Arg Leu Glu Thr Thr Ile Ser Thr
345                 350                 355                 360

GTG GTG GGG GCC CAC GAG GAG GAG CCA GAG GAC GGC CCC AAG GCC ACA 1338
Val Val Gly Ala His Glu Glu Glu Pro Glu Asp Gly Pro Lys Ala Thr
                365                 370                 375

CCC TCG TCC CTG GAC CTG ACC TCC AAC TGC TCT TCA CGA AGT GAC TCC 1386
Pro Ser Ser Leu Asp Leu Thr Ser Asn Cys Ser Ser Arg Ser Asp Ser
            380                 385                 390

AAG ACC ATG ACA GAG AGC TTC AGC TTC TCC TCC AAT GTG CTC TCC     1431
Lys Thr Met Thr Glu Ser Phe Ser Phe Ser Ser Asn Val Leu Ser
        395                 400                 405

TAGGCCACAG GGCCTTTGGC AGGTGCAGCC CCCACTGCCT TTGACCTGCCTCCCTTCATG 1491

CATGGAAATT CCCTTCATCT GGAACCATCA GAAACACCCT CACACTGGGA CTTGCAAAAA 1551

GGGTCAGTAT GGGTTAGGGA AAACATTCCA TCCTTGAGTC AAAAAATCTC AATTCTTCCC 1611

TATCTTTGCC ACCCTCATGC TGTGTGACTC AAACCAAATC ACTGAACTTT GCTGAGCCTG 1671

TAAAATAAAA GGTCGGACCA GCTTTTCCTC AAGAGCCCAA TGCATTCCAT TTCTGGAAGT 1731

GACTTTGGCT GCATGCGAGT GCTCATTTCA GGATG                           1766
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 407 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Asp Asn Val Leu Pro Val Asp Ser Asp Leu Ser Pro Asn Ile Ser
1 5 10 15

Thr Asn Thr Ser Glu Pro Asn Gln Phe Val Gln Pro Ala Trp Gln Ile
20 25 30

Val Leu Trp Ala Ala Ala Tyr Thr Val Ile Val Val Thr Ser Val Val
35 40 45

Gly Asn Val Val Val Met Trp Ile Ile Leu Ala His Lys Arg Met Arg
50 55 60

Thr Val Thr Asn Tyr Phe Leu Val Asn Leu Ala Phe Ala Glu Ala Ser
65 70 75 80

Met Ala Ala Phe Asn Thr Val Val Asn Phe Thr Tyr Ala Val His Asn
85 90 95

Glu Trp Tyr Tyr Gly Leu Phe Tyr Cys Lys Phe His Asn Phe Phe Pro
100 105 110

Ile Ala Ala Val Phe Ala Ser Ile Tyr Ser Met Thr Ala Val Ala Phe
115 120 125

Asp Arg Tyr Met Ala Ile Ile His Pro Leu Gln Pro Arg Leu Ser Ala
130 135 140

Thr Ala Thr Lys Val Val Ile Cys Val Ile Trp Val Leu Ala Leu Leu
145 150 155 160

Leu Ala Phe Pro Gln Gly Tyr Tyr Ser Thr Thr Glu Thr Met Pro Ser
165 170 175

Arg Val Val Cys Met Ile Glu Trp Pro Glu His Pro Asn Lys Ile Tyr
180 185 190

Glu Lys Val Tyr His Ile Cys Val Thr Val Leu Ile Tyr Phe Leu Pro
195 200 205

Leu Leu Val Ile Gly Tyr Ala Tyr Thr Val Val Gly Ile Thr Leu Trp
210 215 220

Ala Ser Glu Ile Pro Gly Asp Ser Ser Asp Arg Tyr His Glu Gln Val
225 230 235 240

Ser Ala Lys Arg Lys Val Val Lys Met Met Ile Val Val Val Cys Thr
245 250 255

Phe Ala Ile Cys Trp Leu Pro Phe His Ile Phe Phe Leu Leu Pro Tyr
260 265 270

Ile Asn Pro Asp Leu Tyr Leu Lys Lys Phe Ile Gln Gln Val Tyr Leu
275 280 285

Ala Ile Met Trp Leu Ala Met Ser Ser Thr Met Tyr Asn Pro Ile Ile
290 295 300

Tyr Cys Cys Leu Asn Asp Arg Phe Arg Leu Gly Phe Lys His Ala Phe
305 310 315 320

Arg Cys Cys Pro Phe Ile Ser Ala Gly Asp Tyr Glu Gly Leu Glu Met
325 330 335

Lys Ser Thr Arg Tyr Leu Gln Thr Gln Gly Ser Val Tyr Lys Val Ser
340 345 350

Arg Leu Glu Thr Thr Ile Ser Thr Val Val Gly Ala His Glu Glu Glu
355 360 365

Pro Glu Asp Gly Pro Lys Ala Thr Pro Ser Ser Leu Asp Leu Thr Ser
370 375 380

Asn Cys Ser Ser Arg Ser Asp Ser Lys Thr Met Thr Glu Ser Phe Ser
385 390 395 400

Phe Ser Ser Asn Val Leu Ser
405

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 12
(D) OTHER INFORMATION: /label= amide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Tyr Arg Pro Lys Pro Gln Gln Phe Phe Gly Leu Met
1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCACCATGGA TAACGTCCTC CCGGTG 26

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTAGGAGAGC ACATTGGAGG AGAA 24

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 407 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Asp Asn Val Leu Pro Met Asp Ser Asp Leu Phe Pro Asn Ile Ser
1               5                  10                  15

Thr Asn Thr Ser Glu Ser Asn Gln Phe Val Gln Pro Thr Trp Gln Ile
            20                  25                  30

Val Leu Trp Ala Ala Ala Tyr Thr Val Ile Val Val Thr Ser Val Val
        35                  40                  45

Gly Asn Val Val Val Ile Trp Ile Ile Leu Ala His Lys Arg Met Arg
    50                  55                  60

Thr Val Thr Asn Tyr Phe Leu Val Asn Leu Ala Phe Ala Glu Ala Cys
65                  70                  75                  80

Met Ala Ala Phe Asn Thr Val Val Asn Phe Thr Tyr Ala Val His Asn
                85                  90                  95

Val Trp Tyr Tyr Gly Leu Phe Tyr Cys Lys Phe His Asn Phe Phe Pro
            100                 105                 110

Ile Ala Ala Leu Phe Ala Ser Ile Tyr Ser Met Thr Ala Val Ala Phe
        115                 120                 125

Asp Arg Tyr Met Ala Ile Ile His Pro Leu Gln Pro Arg Leu Ser Ala
    130                 135                 140

Thr Ala Thr Lys Val Val Ile Phe Val Ile Trp Val Leu Ala Leu Leu
145                 150                 155                 160

Leu Ala Phe Pro Gln Gly Tyr Tyr Ser Thr Thr Glu Thr Met Pro Ser
                165                 170                 175

Arg Val Val Cys Met Ile Glu Trp Pro Glu His Pro Asn Arg Thr Tyr
            180                 185                 190
```

```
Glu Lys Ala Tyr His Ile Cys Val Thr Val Leu Ile Tyr Phe Leu Pro
        195                 200                 205

Leu Leu Val Ile Gly Tyr Ala Tyr Thr Val Val Gly Ile Thr Leu Trp
    210                 215                 220

Ala Ser Glu Ile Pro Gly Asp Ser Ser Asp Arg Tyr His Glu Gln Val
225                 230                 235                 240

Ser Ala Lys Arg Lys Val Val Lys Met Met Ile Val Val Val Cys Thr
                245                 250                 255

Phe Ala Ile Cys Trp Leu Pro Phe His Val Phe Phe Leu Leu Pro Tyr
            260                 265                 270

Ile Asn Pro Asp Leu Tyr Leu Lys Lys Phe Ile Gln Gln Val Tyr Leu
        275                 280                 285

Ala Ser Met Trp Leu Ala Met Ser Ser Thr Met Tyr Asn Pro Ile Ile
    290                 295                 300

Tyr Cys Cys Leu Asn Asp Arg Phe Arg Leu Gly Phe Lys His Ala Phe
305                 310                 315                 320

Arg Cys Cys Pro Phe Ile Ser Ala Gly Asp Tyr Glu Gly Leu Glu Met
                325                 330                 335

Lys Ser Thr Arg Tyr Leu Gln Thr Gln Ser Ser Val Tyr Lys Val Ser
            340                 345                 350

Arg Leu Glu Thr Thr Ile Ser Thr Val Val Gly Ala His Glu Glu Glu
        355                 360                 365

Pro Glu Glu Gly Pro Lys Ala Thr Pro Ser Ser Leu Asp Leu Thr Ser
    370                 375                 380
```

Asn Gly Ser Ser Arg Ser Asn Ser Lys Thr Met Thr Glu Ser Ser Ser
385 390 395 400

Phe Tyr Ser Asn Met Leu Ala
405

WHAT IS CLAIMED IS:

1. A recombinant DNA sequence comprising a sequence encoding human substance P receptor protein having the amino acid sequence shown in FIG. 1.

2. Human substance P receptor cDNA having the nucleotide sequence shown in FIG. 1.

3. A process which comprises expressing a cDNA encoding human substance P receptor having the amino acid sequence shown in FIG. 1 in a bacterial or mammalian cell culture transformed with a DNA expression vector containing said gene operably linked to transcription and translation sequences in said vector and recovering said human substance P receptor.

4. The process of Claim 3 in which the DNA expression vector is plasmid pM^2hSPR.

5. CHO cells transformed with a DNA expression vector containing a cDNA encoding human substance P receptor having the amino acid sequence shown in FIG. 1 operably linked to transcription and translation sequences in said vector.

6. The cells of Claim 5 in which the DNA expression vector is plasmid pM^2hSPR.

7. Plasmid pM^2hSPR.

8. Cell line CHO-pM^2hSPR #10 (ATCC CRL 10824).

FIG. 1

```
                (-210)AATTCAGAGCCACCGCGGGGCAGGCGGGCAGTGCATCCAGAAGCGTTTATATTCTGAGCGCCCAGTTCAGCTTTCAAAAAGAGTGCTGCCCATAAAAA  -115

GCCTTCCACCCTCCTGTCTGCTTTAGAAGGACCCTGAGCCCCAGGCGCCAGCCACAGGACTCTGGTGCAGAGGGGGGTTGTGTACAGATAGTAGGCTTTACGCCTAGCTTCGAA    -1

ATGGATAACGTCCTCCCCGGTGGACTCAGACCTCTCCCCAAACATCTCCACTAACACCTCGGAACCCAATCAGTTCGTGCAACCAGCCTCGCAAATTGTCCTTTGGGCAGCTGCC  +114
MetAspAsnValLeuProValAspSerAspLeuSerProAsnIleSerThrAsnThrSerGluProAsnGlnPheValGlnProAlaTrpGlnIleValLeuTrpAlaAlaAla
1                                                  21                                                  MI
TACACGGTCATTGTGGTGACCTCTGTGGTGGGCAACGTGGTAGTGATGTCGATCATCTTAGCCCACAAAAGAATGAGGACAGTGACGAACTATTTTCTGGTGAACCTGGCCTTC  +228
TyrThrValIleValValThrSerValValGlyAsnValValValMetTrpIleIleLeuAlaHisLysArgMetArgThrValThrAsnTyrPheLeuValAsnLeuAlaPhe
  41                                                  61                                                  MII
GCGGAGGCCTCCATGCCTGCATTCAATACAGTGGTGAACTTCACCTATGCTGTCCACAACGAATGGTACTACGGCCTGTTCTACTGCAAGTTCCACAACTTCTTTCCCATCGCC  +342
AlaGluAlaSerMetAlaAlaPheAsnThrValValAsnPheThrTyrAlaValHisAsnGluTrpTyrTyrGlyLeuPheTyrCysLysPheHisAsnPhePheProIleAla
    81                                                 101                                                 MIII
GCTGTCTTCGCCAGTATCTACTCCATGACGGCTGTGGCCTTTGATAGGTACATGGCCATCATACATCCCCTCCAGCCCCCCCTGTCAGCCACAGCCACCAAAGTGGTCATCTGT  +456
AlaValPheAlaSerIleTyrSerMetThrAlaValAlaPheAspArgTyrMetAlaIleIleHisProLeuGlnProArgLeuSerAlaThrAlaThrLysValValIleCys
                  121                                                 141                                 MIV
GTCATCTGGGTCCTGGCTCTGCTGGCCTTCCCCCAGGGCTACTACTCAACCACAGAGACCATGCCCAGCAGAGTCGTGTGCATGATCCAATGGCCAGAGCATCCGAACAAG  +570
ValIleTrpValLeuAlaLeuLeuLeuAlaPheProGlnGlyTyrTyrSerThrThrGluThrMetProSerArgValValCysMetIleGluTrpProGluHisProAsnLys
                  161                                                 181
ATTTATGAGAAAGTGTACCACATCTGTGTGACTGTGCTGATCTACTTCCTCCCCCTGCTGGTGATTGGCTATGCATACACCGTAGTGGCAATCACACTATGGGCCAGTGAGATC  +684
IleTyrGluLysValTyrHisIleCysValThrValLeuIleTyrPheLeuProLeuLeuValIleGlyTyrAlaTyrThrValValGlyIleThrLeuTrpAlaSerGluIle
      MV          201                                                 221
CCCGGGGACTCCTCTGACCCCTACCACGAGCAAGTCTCTGCCAAGCGCAAGGTGGTCAAAATGATGATTGTCGTGGTGTGCACCTTCGCCATCTGCTGGGTGCCCTTCCACATC  +798
ProGlyAspSerSerAspArgTyrHisGluGlnValSerAlaLysArgLysValValLysMetMetIleValValValCysThrPheAlaIleCysTrpLeuProPheHisIle
                  241                                 MVI                                 261
TTCTTCCTCCTGCCCTACATCAACCCAGATGTCTACGTGAAGAAGTTTATCCAGCAGGTCTACCTGGCCATCATGTGGCTGGCCATGAGCTCCACCATGTACAACCCCATCATC  +912
PheLeuLeuProTyrIleAsnProAspLeuTyrLeuLysLysPheIleGlnGlnValTyrLeuAlaIleMetTrpLeuAlaMetSerSerThrMetTyrAsnProIleIle
                  281                                 MVII                                301
TACTGCTGCCTCAATGACAGGTTCCGTCTGGGCTTCAAGCATGCCTTCCGGTGCTGCCCCTTCATCAGCGCCGCGCGACTATGAGGGCCTGGAAATGAAATCCACCCGGTATCTC  +1026
TyrCysCysLeuAsnAspArgPheArgLeuGlyPheLysHisAlaPheArgCysCysProPheIleSerAlaGlyAspTyrGluGlyLeuGluMetLysSerThrArgTyrLeu
                  321                                                                                 341
CAGACCCAGGGCAGTGTGTACAAAGTCAGCCGCCTGGAGACCACCATCTCCACAGTGGTCGGGGGCCCACGAGGAGGAGCCAGAGGACGGCCCCAAGGCCACACCCTCGTCCCTG  +1140
GlnThrGlnGlySerValTyrLysValSerArgLeuGluThrThrIleSerThrValValGlyAlaHisGluGluGluProGluAspGlyProLysAlaThrProSerSerLeu
                  361
GACCTGACCTCCAACTGCTCTTCACGAAGTGACTCCAAGACCATGACAGAGAGCTTCAGGTTCTCGTCGCAATGTGCTCTCGTAGGCCACAGGGCCTTTGGCAGGTCCACCCCCC  +1254
AspLeuThrSerAsnCysSerSerArgSerAspSerLysThrMetThrGluSerPheSerPheSerSerAsnValLeuSerEnd
381                                                 401                 407
ACTGCCTTTGACCTGCCTCCCTTCATGCATGGAAATTCCCTTCATCTGGAACGATCAGAAACACCCTCACACTGCGACTTGCAAAAAGGGTCAGTATGCGTTAGGCAAAACATT  +1368

CCATCCTTGAGTCAAAAAATCTCAATTCTTCCCTATCTTTGCCACCCTCATGCTGTGTGACTCAAACCAAATCACTGAACTTTGCTCAGCCTGTAAAATAAAAGGTCCGCACCAG  +1482

CTTTTCCTCAAGAGCCCAATGCATTCCATTTCTGGAAGTGACTTTGGCTGCATGCGAGTGCTCATTTCAGGATG  +1556
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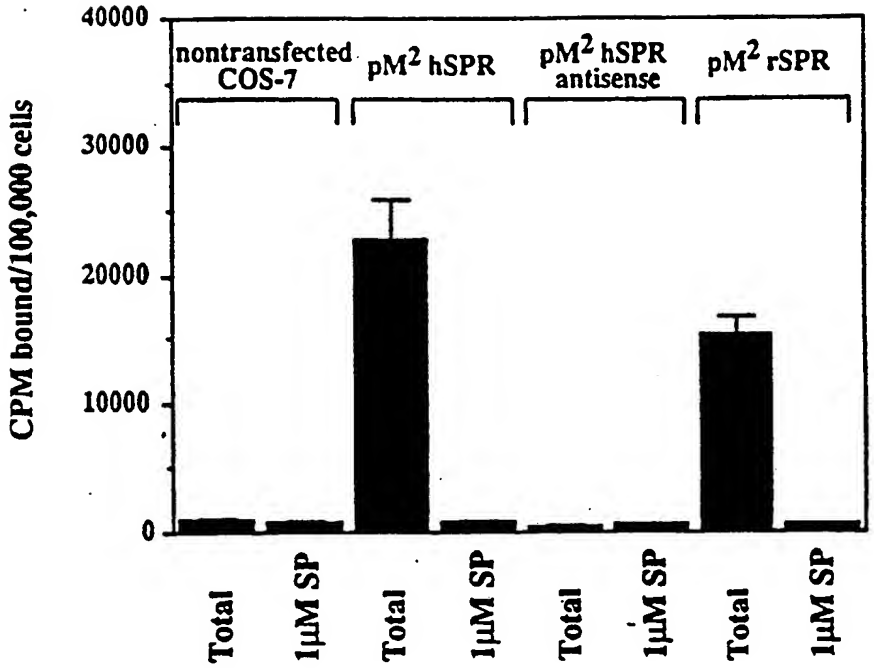
40000
30000
20000
10000
0
CPM bound/100,000 cells
nontransfected COS-7
pM2 hSPR
pM2 hSPR antisense
pM2 rSPR
Total
1μM SP
Total
1μM SP
Total
1μM SP
Total
1μM SP

FIG. 2A

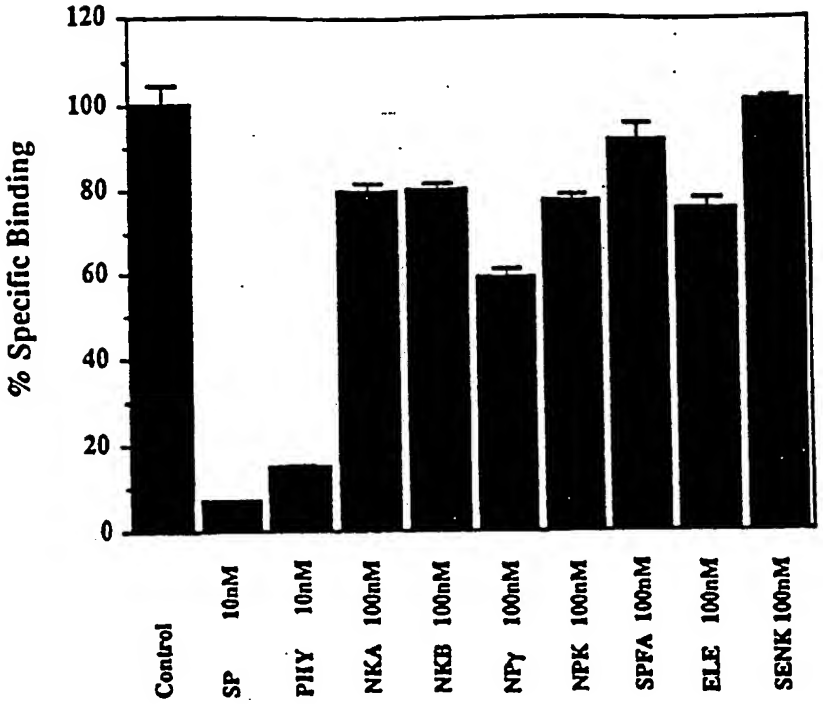
% Specific Binding
120
100
80
60
40
20
0
Control
SP 10nM
PHY 10nM
NKA 100nM
NKB 100nM
NPγ 100nM
NPK 100nM
SPFA 100nM
ELE 100nM
SENK 100nM

FIG. 2B

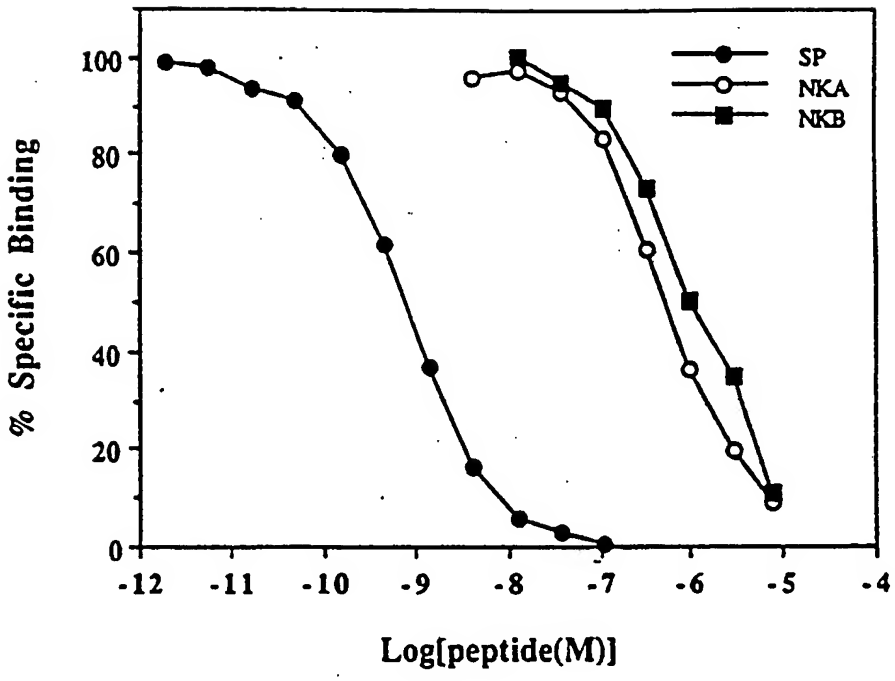
% Specific Binding
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
-4
Log[peptide(M)]
SP
NKA
NKB

FIG-3

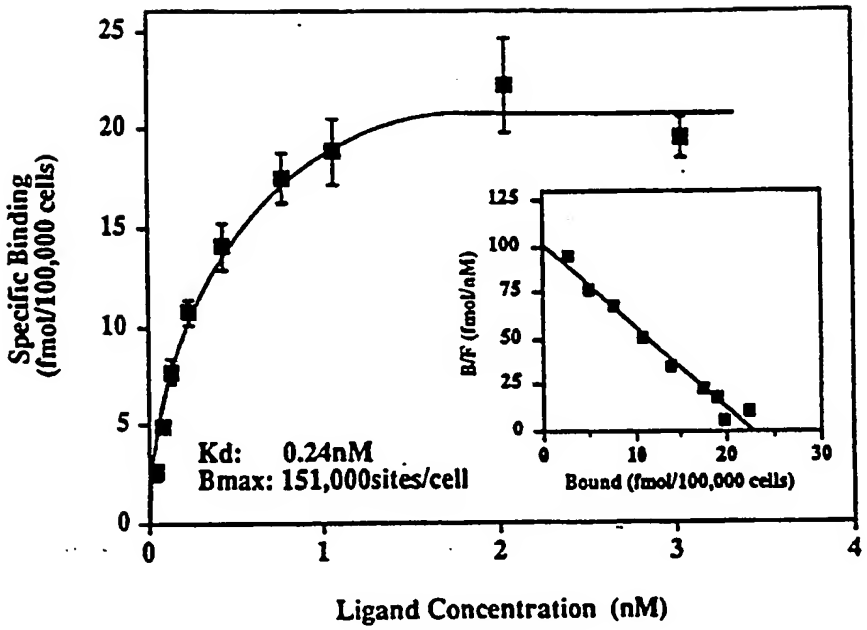
Specific Binding
(fmol/100,000 cells)
25
20
15
10
5
0
0
1
2
3
4
Ligand Concentration (nM)
Kd: 0.24nM
Bmax: 151,000sites/cell
B/F (fmol/nM)
125
100
75
50
25
0
0
10
20
30
Bound (fmol/100,000 cells)

FIG. 4

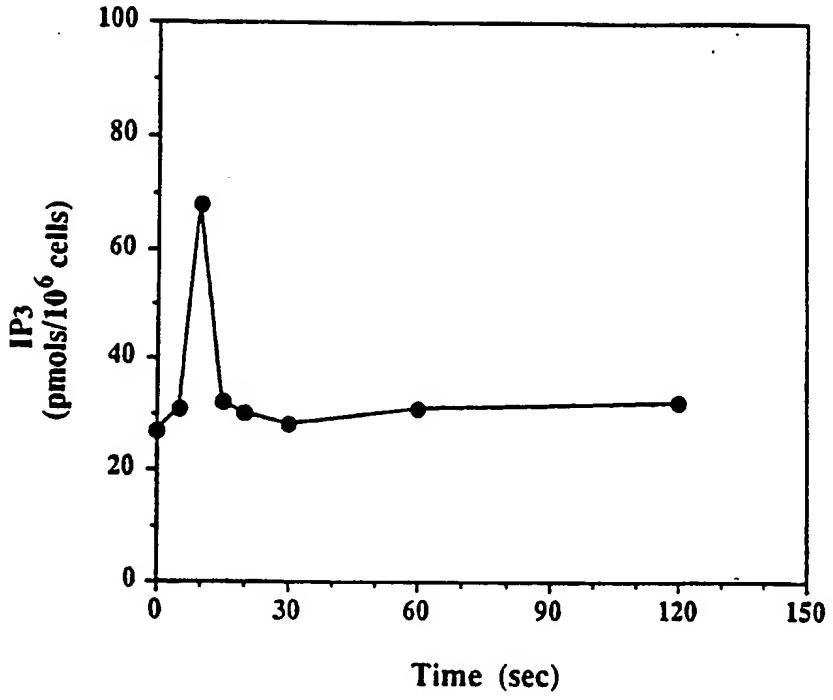

FIG. 5

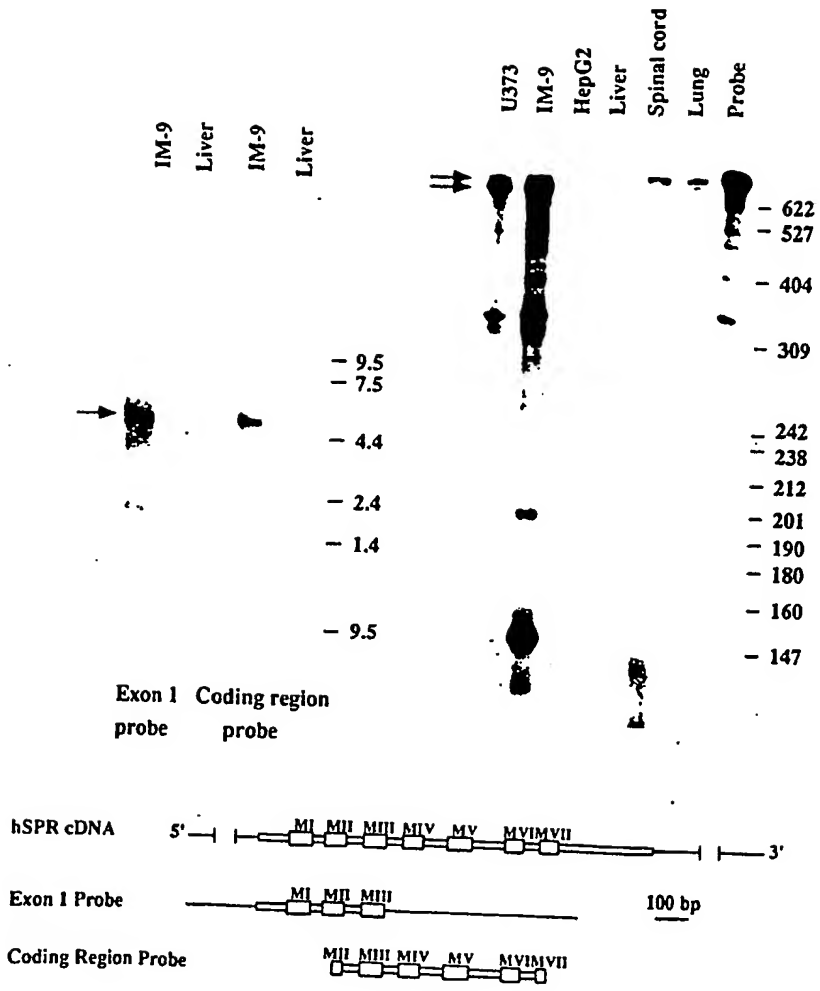
IM-9
Liver
IM-9
Liver
U373
IM-9
HepG2
Liver
Spinal cord
Lung
Probe
622
527
404
309
242
238
212
201
190
180
160
147
9.5
7.5
4.4
2.4
1.4
9.5
Exon 1 probe
Coding region probe
hSPR cDNA
5'
MI MII MIII MIV MV MVIMVII
3'
Exon 1 Probe
MI MII MIII
100 bp
Coding Region Probe
MII MIII MIV MV MVIMVII

FIG. 6

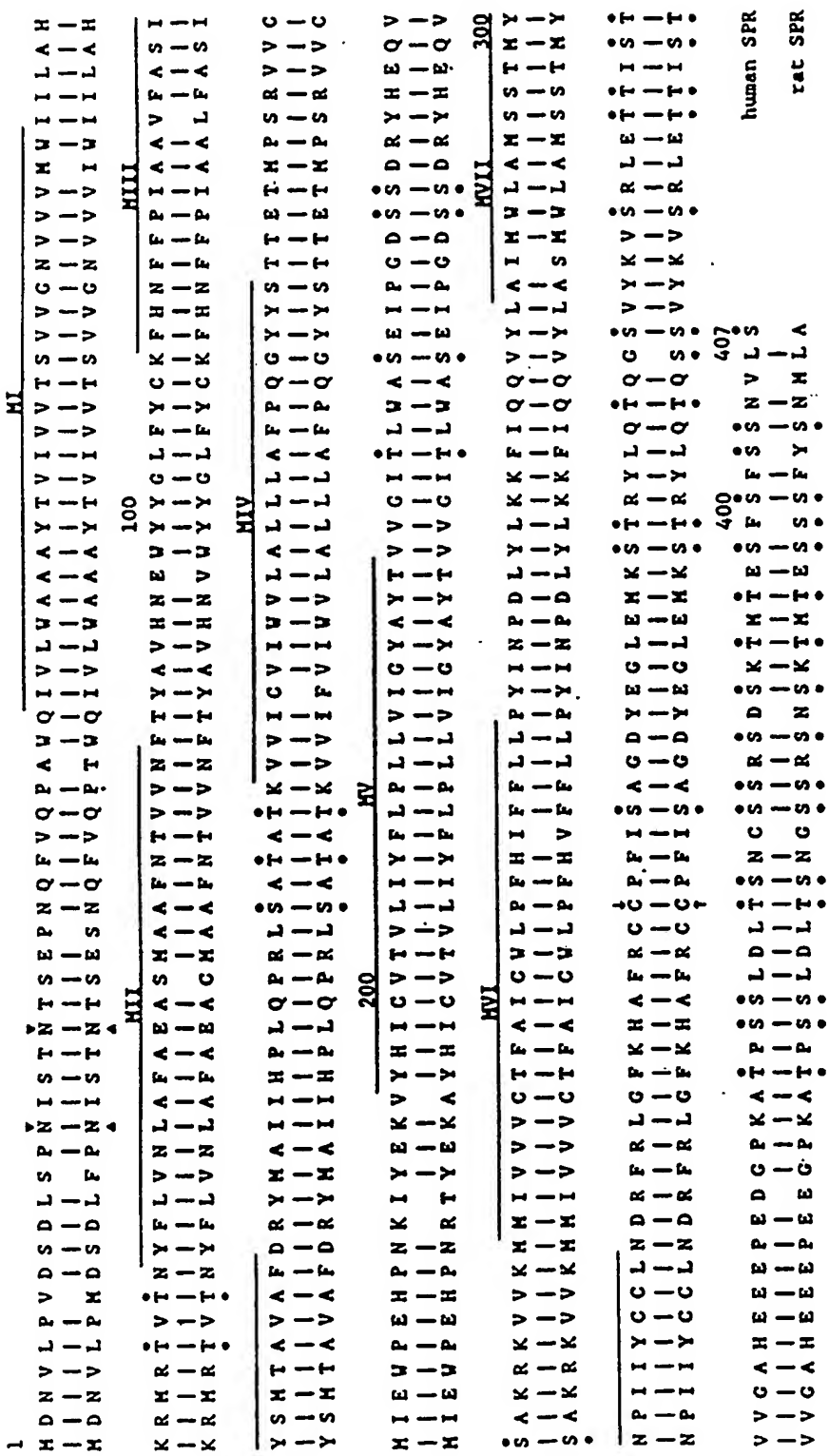

FIG. 7

INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/US92/06532

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12N 1/20, 5/00, 15/00; C12P 19/34, 21/06; C12Q 1/68
US CL :435/6, 69.1, 91, 172.3, 240.2, 252.3, 320.1; 536/27
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 69.1, 91, 172.3, 240.2, 252.3, 320.1; 536/27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, INTELLIGENETICS, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y.	BIOCHEMISTRY, Volume 30, No. 44, issued 1991, N. P. Gerard et al, "Human Substance P Receptor(NK-1): Organization of the Gene, Chromosome Localization, and Functional Expression of cDNA Clones", pages 10640-10646, see entire document.	1-3 4-8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 OCTOBER 1992	Date of mailing of the international search report 18 NOV 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer LAURIE SCHEINER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*